Kidney International, Vol. 67 (2005), pp. 2305–2312

Validation of quantitative BOLD MRI measurements in kidney: Application to unilateral ureteral obstruction

MICHAEL PEDERSEN, THOMAS H. DISSING, JAN MØRKENBORG, HANS STØDKILDE-JØRGENSEN, LARS H. HANSEN, LARS B. PEDERSEN, NICOLAS GRENIER, and JØRGEN FRØKIÆR

MR Research Centre, Clinical Institute, Aarhus University, Aarhus, Denmark; Department of Clinical Physiology, Aarhus University Hospital, Skejby, Aarhus, Denmark; Unisense Aps., Aarhus Science Park, Aarhus, Denmark; and Universite Victor Segalen-Bordeaux 2, Bordeaux, France

Validation of quantitative BOLD MRI measurements in kidney: Application to unilateral ureteral obstruction.

Background. Blood oxygenation level dependent (BOLD) magnetic resonance imaging (MRI) provides a measure of deoxyhemoglobin content and therefore an indirect measure of the partial oxygen pressure (pO_2). The main purpose of this study was to examine the relationship between the apparent relaxation rate ($R2^*$) in the pig kidney by BOLD imaging and renal tissue pO_2 levels measured directly by oxygen-sensitive microelectrodes. Second, BOLD imaging was applied to kidneys in pigs subjected to acute unilateral ureteral obstruction (UUO) to examine whether this condition is associated with changes in intrarenal oxygenation.

Methods. Oxygen-sensitive microelectrodes were inserted in the cortex and medulla of pig kidneys ($N = 6$). Different arterial and intrarenal levels of pO_2 were obtained by stepwise changing the oxygen-to-nitrogen ratio supplied by a respirator. Simultaneous BOLD MRI measurements using an $R2^*$-sensitive Echo Planar Imaging (EPI) sequence were performed on the contralateral kidney. In another group of pigs ($N = 3$) BOLD imaging was performed following 24 hours of UUO.

Results. When the inhaled oxygen fraction was 5% to 70%, $R2^*$ was linearly related to pO_2 levels (cortex $\Delta R2^*/\Delta pO_2 = -1.2\ ms^{-1}kPa^{-1}$, and medulla $\Delta R2^*/\Delta pO2 = -1.7\ ms^{-1}kPa^{-1}$). Twenty-four hours of UUO was associated with an increased $R2^*$ in the cortex and a decreased $R2^*$ in medulla as compared with baseline, which remained augmented after the release of UUO, indicating that pO_2 levels were reduced in the cortex and increased in the medulla during and after release of obstruction.

Conclusion. BOLD MRI provides noninvasive estimates of regional renal oxygen content and our study demonstrates that this technique may provide a useful tool in UUO which is associated with altered renal oxygen consumption.

Key words: blood oxygenation level dependent MRI, obstructive nephropathy, kidney tubules, pigs.

Received for publication March 10, 2004
and in revised form October 18, 2004, and December 13, 2004
Accepted for publication January 11, 2005

Blood oxygenation level dependent (BOLD) magnetic resonance imaging (MRI) was in this study applied to experimental conditions, where the underlying physiologic changes suggest altered renal oxygen consumption. The interaction between renal medullary transport of sodium and water and oxygen consumption supports that measurement of partial pressure of oxygen (pO_2) in the kidney is an important parameter when renal function is impaired. Precise methods for regional measurements of pO_2 in the renal tissue involve direct insertion of oxygen sensitive microelectrodes, whereas techniques such as near-infrared spectroscopy, nuclear magnetic resonance spectroscopy, and electron paramagnetic resonance allow indirect measurement of the in vivo concentrations of oxygen [1–6]. However, different restrictions and physical requirements make these methods currently unavailable for clinical purposes. BOLD MRI is another noninvasive technique, which has shown capable of providing indirect measures of the renal oxygen content in vivo [7]. This technique is based on diamagnetic properties of oxyhemoglobin whereas deoxyhemoglobin has paramagnetic properties in a magnetic field [8]. Changes in deoxyhemoglobin concentration involve generation of phase incoherence of magnetic spins, leading to signal attenuation in gradient echo sequences and an increase in the apparent spin-spin relaxation rate denoted as $R2^*$. This behavior, combined with the fact that pO_2 in blood is in equilibrium with pO_2 in tissue, potentates BOLD MRI to be an attractive tool for high resolution mapping of the intrarenal oxygenation. Unfortunately, the BOLD technique does not allow quantifying pO_2 in a tissue directly. It is, however, generally assumed that the change of $R2^*$ is closely related to the change of pO_2 [7], implying that values of $\Delta R2^*$ can be interpreted as measures of ΔpO_2 if conversion constants are known; although intrinsically dependent on renal blood flow and renal blood volume.

In the kidney the majority of the renal energy expenditure, estimated from its oxygen consumption, is devoted to the reabsorption of electrolytes filtered into the

tubules. Thus, the local oxygen tension within the kidney tissue reflects the balance between oxygen delivery and consumption of oxygen in viable cells and tissue. The renal medulla has a relatively small blood flow to support the active work of sodium transport via the activity of basolateral sodium, potassium-adenosine triphosphatase (Na^+, K^+-ATPase), and since the renal oxygen consumption has been shown to linearly correlate with the sodium transport [9], lack of oxygen obviously affects this function critically. In view of this fact, it is interesting that the renal medulla exists on the edge of hypoxia [10]. Although the cortical pO_2 has consistently been shown to be higher than that in the medulla [11], the renal cortex may also be susceptible to hypoxia since the cortex is unable to extract sufficient oxygen unless the concentration of oxygen is relatively high [12].

Unilateral ureteral obstruction (UUO) is well-known to be associated with reductions in renal blood flow (RBF), glomerular filtration rate (GFR) and pO_2 in the renal parenchyma, providing the basis for development of obstructive nephropathy [13]. In parallel, renal handling of water and solutes is compromised, leading to impaired urinary concentrating capacity [13]. These metabolic and hemodynamic changes may in part be explained by alterations in the expression of renal prostaglandins and activation of the intrarenal renin-angiotensin system [14–17]. The renin-angiotensin-aldosterone system has been demonstrated to play a critical role in the regulation of sodium and water metabolism through a variety of physiologic pathways. In addition to the hemodynamic effects, angiotensin II may have direct effects on sodium transport in the proximal tubule [18, 19]. Thus, a better understanding of the changes in renal oxygen content during and after release of obstruction may provide a useful avenue to determine when obstruction of the ureter impairs renal functions.

The purposes of the present feasibility study were threefold. First, the relationship between $\Delta R2^*$ measured by BOLD MRI and ΔpO_2 measured by oxygen-sensitive microelectrodes was investigated. Second, BOLD MRI was performed following administration of furosemide to investigate the effect of loop diuretics on the renal tissue oxygenation. Third, BOLD MRI was performed during and after release of UUO to examine whether changes in intrarenal oxygenation associated with this condition can be detected.

METHODS

Animal handling

Twelve female Danish Landrace pigs were included in the study. The animals were kept on a standard pig diet and had free access to food and water until the start of the experiment. The pigs were preanesthetized using 10 mg/kg ketaminol (Veterinaria, Zurich, Switzerland) and 5 mg/kg dormicum (Dumex-Alpharma, Oslo, Norway). Following orotracheal intubation, general anesthesia was maintained with 1.2% isoflurane during surgical procedures. During MRI, anesthesia was accomplished by intravenously infusion of dormicum (5 μg/min/kg), ketaminol (0.3 mg/min/kg), and pancurone (2.5 μg/min/kg) (Organon Teknika, Boxtel, The Netherlands). A dedicated MRI-compatible respirator supplied with two gas flasks containing 100% O_2 and 100% N_2, respectively, maintained artificial ventilation. Each pig received 0.1 mL/min/kg of isotonic saline intravenously throughout the study. Animals were divided into the following protocols.

Protocol 1: Relationship between $R2^*$ and pO_2 in renal tissue ($N = 6$). A catheter was inserted in a femoral artery to allow blood sampling for arterial pO_2 monitoring. One kidney was exposed retroperitoneally by a deep flank incision and immobilized by attaching the peritoneum to the flank with sutures. Oxygen-sensitive microelectrodes were inserted and placed in the central cortical and medullary regions of the kidney guided directly from the measured pO_2 level (i.e., a high pO_2 level indicated that the microelectrode was in the renal cortex and a low pO_2 level indicated that the tip of the microelectrode was in the renal medulla). The body temperature was maintained by surrounding the body with insulating blankets. During the experimental procedure, different arterial and intrarenal levels of pO_2 were achieved by sequentially changing the percentage of the respirator supplied oxygen-nitrogen fraction. The arterial pO_2 levels in the blood samples were analyzed (ABL555) (Radiometer, Copenhagen, Denmark). A decrease in the supply of O_2 may consequently reduce the concentration of saturated hemoglobin. Each pig was subjected to four to five different levels of oxygen concentrations, and the range of O_2 was adjusted from a normoxic/hyperoxic level (O_2 = 70%) to a hypoxic level (O_2 = 5%). All adjustments in the O_2 concentrations were made in a descending order and each change in O_2 concentration was followed by a 20-minute period of rest before additional reduction in O_2 concentration. Measurements of pO_2 and BOLD MRI were performed in an interleaved order. To avoid artefacts induced by the oxygen microelectrode, imaging was performed in the opposite kidney with the assumption that the physiologic conditions of the two kidneys were comparable during the time of examination.

Protocol 2: Measurement of $R2^*$ in response to furosemide ($N = 3$). The effect of loop diuretics on the renal oxygenation was investigated by measuring intrarenal $R2^*$ before and after an intravenous bolus (0.5 mg/kg) of furosemide (Hoechst-Roussel Pharmaceuticals, Kansas City, MO, USA).

Protocol 3: Measurement of $R2^*$ in response to UUO and release of UUO ($N = 3$). Twenty-four hours prior to MRI, pigs were operated upon in general anesthesia.

Using sterile procedures, a subcostal flank incision was made and the left ureter was isolated. UUO was induced by carefully cannulating the left ureter and placing the tip of a catheter in the renal pelvis. The catheter was left in place with the other end of the catheter closed using a stop-gauge. After 24 hours of UUO, measurements of intrarenal R2* in both the obstructed and contralateral nonobstructed kidney were performed. Approximately 2 hours after this, the obstruction was released by removal of the stop-gauge, and urine was allowed to drain through the catheter. Measurement of R2* was repeated 1 hour after release of UUO.

Oxygen electrodes

Dedicated Clark-type oxygen sensitive electrodes were used for direct measurement of the intrarenal pO_2 (Unisense Aps, Aarhus, Denmark) [3]. The electrode tip has a diameter of 200 μm, which allows diffusion of oxygen through a silicone membrane to an oxygen-reducing cathode polarized against an internal Ag/AgCl anode (−0.8 V). The flow of electrons from the anode to the cathode corresponds to the pO_2 around the electrode tip. The sensor was connected to a high-sensitivity picoammeter (Unisense PA2000) and the cathode was polarized against an internal reference. The oxygen sensor responded linearly in the range of 0% to 100% oxygen, allowing linear conversion of output currents into measures of pO_2. The detection limit was as low as 0.1 μmol/L of oxygen. All devices were initially checked for any MRI incompatibility, and on the day of the experimental procedure, the microelectrodes were calibrated in oxygen-free medium (0.1 mol/L sodium ascorbate).

MRI protocol

MRI was performed on a 1.5 Tesla clinical system (GE Medical Systems, Milwaukee, WI, USA). The system was equipped with gradient capacity enabling a gradient strength of 23 mT/m and a slewrate of 120 mT/m/second. Animals were placed supine in a cylindric birdcage radio frequency coil with a length of 415 mm and an inner diameter of 275 mm. Sagittal and coronal slices were initially obtained to localize regions of interest. BOLD MRI was performed using a single-shot gradient-echo echo-planar sequence in the transaxial plane. Acquisition parameters included recovery time of 2400 msec, 90° radiofrequency excitation flip-angle, 100 kHz signal bandwidth, 128 ± 128 acquisition matrix, and 30 ± 30 cm^2 field-of-view. Six echo times (TE) were equidistantly applied in the range from 40 to 140 msec. To optimize the signal-to-noise ratio each acquisition was repeated eight times. The image of each TE value was acquired during a temporary respiration stop controlled by the ventilator (∼18 seconds), followed by 75 seconds of rest to recover for differences in the cortical and medullary oxygen concentration induced by the respiratory stop. MRI data were transferred to an external computer, and regions of medulla and cortex were manually placed using homemade analysis software. Determination of R2* [equal to the slope of the Ln (signal intensity) versus TE curve] was performed using standard least-square fitting.

Statistics

The relationships of data measured by BOLD MRI, microelectrodes, and blood gas analyses were evaluated by scatterplots and Pearson's product moment correlation coefficient (r). Tests for linearity were statistically evaluated by a first-order equation null hypothesis. The effect of furosemide was evaluated using a paired two-tailed Student t test. Although the number of animals was limited, statistical differences between pO_2 of UUO kidneys were performed by a one-way analysis of variance (ANOVA). Statistical differences between ipsilateral pO_2 and contralateral pO_2 were evaluated using a two-sample paired Student t test. Statistics were performed by the SAS statistical package (SAS Institute Inc, Cary, NC, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Measurement of R2* by BOLD MRI correlated linearly with pO_2 levels in the renal tissue

In protocol 1, we examined the relationship between pO_2 levels measured by gas analysis of arterial blood and renal cortical and medullary pO_2 levels measured directly with the oxygen sensing microelectrodes. Linear regression was performed using the assumption that the slope must intercept at origin, since oxygen presence in the kidney is possible only during the presence of oxygen in arterial blood. Arterial pO_2 levels correlated linearly with both cortical pO_2 levels (0.30 ± 0.02) ($r = 0.90$) and medullary pO_2 levels (0.22 ± 0.01) ($r = 0.94$) (Fig. 1).

Next, we examined if there exists a relationship between the R2* measured by BOLD MRI and renal tissue pO_2 levels. Cortical and medullary R2* levels correlated linearly with renal tissue pO_2 levels (Fig. 2). From these data a fitted first-order linear equation was generated for the cortex (21.1 $msec^{-1}$ − $pO_2 \cdot 1.2$ $msec^{-1}kPa^{-1}$) ($r = 0.73$) as well as for the medulla (25.7 $msec^{-1}$ − $pO_2 \cdot 1.7$ $msec^{-1}kPa^{-1}$) ($r = 0.67$). Linear regression was statistically accepted ($P < 0.001$).

The effect of furosemide on renal oxygenation

The effect of intravenous administration of furosemide on R2* is shown in Figure 3A. The changes in R2* were quantitatively interpreted in terms of kPa units using the equation derived in protocol 1. Furosemide administration was associated with moderately decreased R2* in

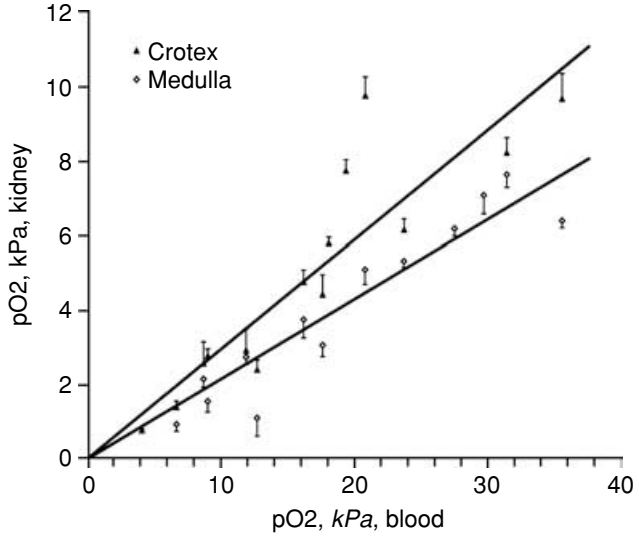

Fig. 1. Partial pressure of oxygen (pO_2) in the renal cortex and medulla measured by oxygen-sensitive microelectrodes were linearly related to pO_2 in arterial blood measured by a gas analysis. Mean values are shown with standard error of the mean.

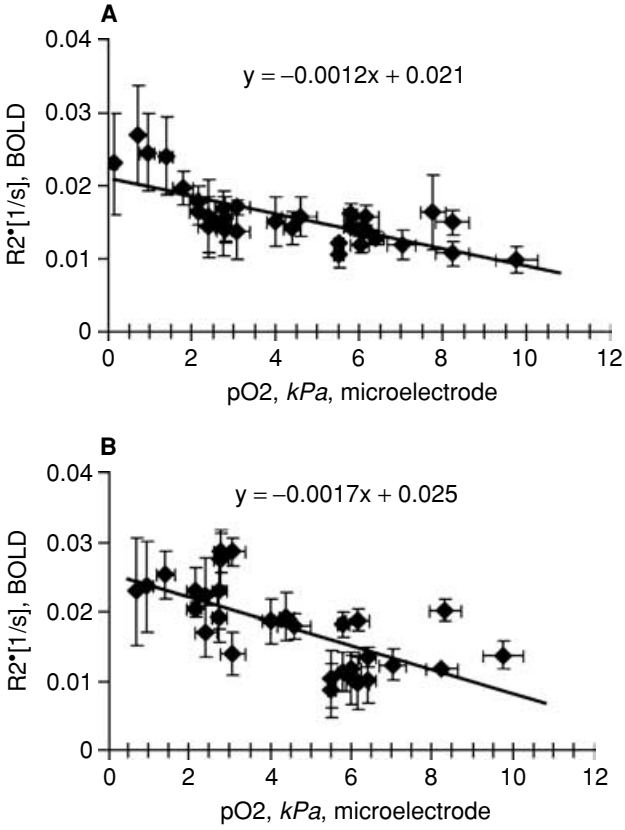

Fig. 2. The graphs demonstrate the relationship between spin-spin relaxation rates (R2*) and pO_2. Data include standard error of mean of cortical (*A*) and medullary (*B*) measurements. The solid line represents the linear regression to the data.

the cortex (from 12.7 ± 0.6 msec^{-1} to 11.3 ± 0.6 msec^{-1}), corresponding to a statistically significant increase in the pO_2 level ($\Delta pO_2 = 1.2 \pm 0.5$ kPa) ($P = 0.015$). Medullary R2* decreased markedly in response to furosemide (from 18.5 ± 0.6 msec^{-1} to 12.5 ± 1.2 msec^{-1}), corresponding to a statistically significant increase in the pO_2 level in the renal medulla ($\Delta pO_2 = 3.5 \pm 0.7$ kPa) ($P < 0.001$).

UUO and release of UUO are associated with changes in intrarenal R2*

After 24 hours of UUO R2* values in the cortex of the obstructed kidney were significantly increased compared with baseline R2* values obtained in normal untreated kidneys (Fig. 3B and C) (18.8 ± 1.3 msec^{-1} vs. 12.7 ± 0.6 msec^{-1}) ($P < 0.001$). In contrast, R2* in the renal medulla of the obstructed kidney was slightly decreased (14.9 ± 1.8 msec^{-1} vs. 18.5 ± 0.6 msec^{-1}) (NS) suggesting that UUO causes a reduction in the pO_2 levels in the cortex and a slight increase in the pO_2 levels in the medulla of the obstructed kidney after 24 hours of UUO. Twenty-four hours of UUO did not reveal marked changes between the ipsilateral and contralateral R2* neither in the cortex ($R2^* = 18.8 \pm 1.3$ msec^{-1} vs. $R2^* = 16.2 \pm 0.8$ msec^{-1}) (NS) nor in the medulla ($R2^* = 14.9 \pm 1.8$ msec^{-1} vs. $R2^* = 13.4 \pm 0.6$ msec^{-1}) (NS). After release of UUO, cortical R2* decreased slightly in the ipsilateral kidney (from 18.8 ± 1.3 msec^{-1} to 17.1 ± 1.5 msec^{-1}), corresponding to an increase in pO_2 ($\Delta pO_2 = 1.0 \pm 2.5$ kPa) (NS), whereas cortical R2* increased slightly in the contralateral kidney (from 16.2 ± 0.8 msec^{-1} to 18.5 ± 1.0 msec^{-1}), corresponding to a slight decrease in pO2 ($\Delta pO2 = -1.9 \pm 1.9$ kPa; N.S.). In addition, release of UUO revealed that medullary R2* was unchanged in the ipsilateral kidney (from 14.9 ± 1.8 msec^{-1} to 14.5 ± 1.9 msec^{-1}) ($\Delta pO_2 = 0.2 \pm 0.9$ kPa) (NS). In the contralateral kidney R2* increased moderately (from 13.4 ± 0.6 msec^{-1} to 16.3 ± 2.5 msec^{-1}) ($\Delta pO_2 = -1.7 \pm 1.6$ kPa) (NS).

DISCUSSION

The present study confirmed a linear relationship between measurement of R2* in the kidney by BOLD MRI and direct measurements of pO_2 levels in the renal tissue using oxygen sensitive microelectrodes. This finding demonstrates that BOLD MRI provides a reliable estimate of regional renal oxygen content. Intravenous administration of furosemide was associated with an acute R2* decrease in both the renal cortex and medulla demonstrating the anticipated reduction in the metabolic demands in response to diuretics. Twenty-four hours of UUO release were associated with an increased R2* in cortex in parallel with a slightly decreased R2* in the renal medulla, which persisted after release of UUO, suggesting that the pO_2 levels in cortex are reduced, whereas the

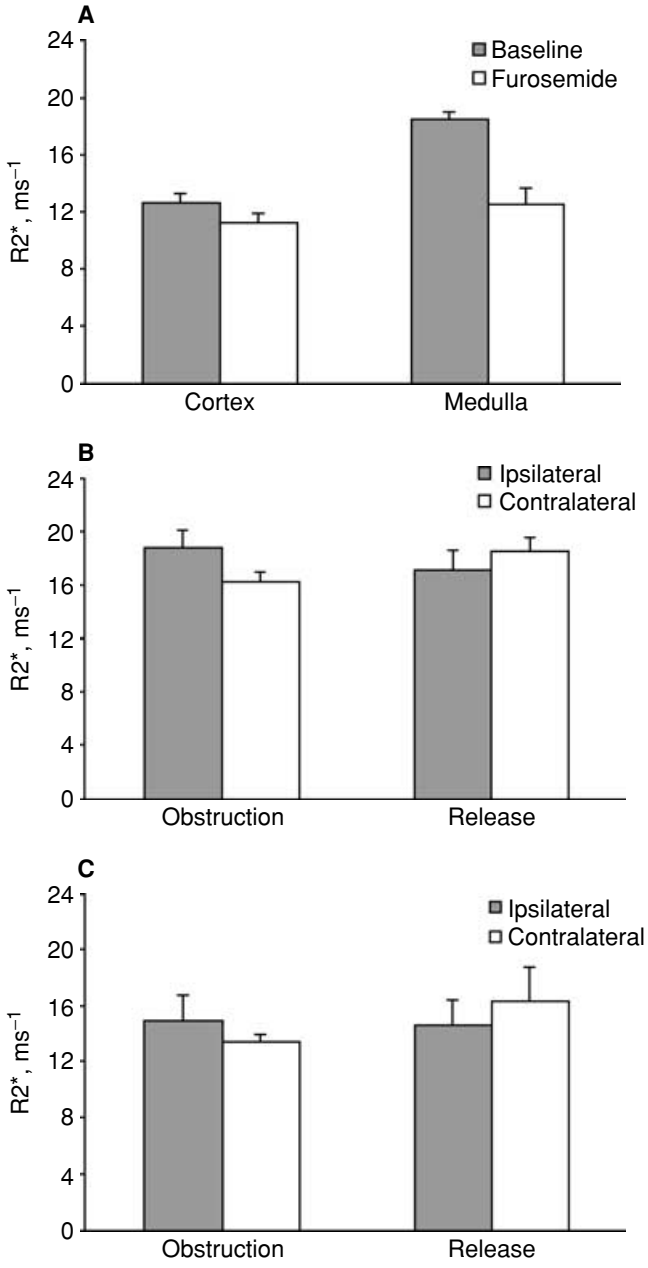

Fig. 3. The effect of furosemide administration (protocol 2) on R2* in the renal cortex and the renal medulla (*A*), demonstrating an increased oxygenation level following furosemide. Measurements of R2* in response to unilateral ureteral obstruction (UUO) (protocol 3) are shown for the cortex (*B*) and in the medulla (*C*) before and after release of 24 hours of UUO. Data are shown as mean values with standard error of mean.

pO_2 levels in the renal medulla are increased in response to acute obstruction.

BOLD MRI is sensitive to oxygenation

Although the kidney receives approximately a quarter of the cardiac output and has the highest organ blood flow in the body, this large blood supply is mainly directed to the cortex to optimize glomerular filtration and reabsorption of solutes. By contrast, the blood flow to the medulla is low, and generally, the medulla is poorly oxygenated, resulting in a medullary pO_2 consistently lower than that in the cortex. The MRI BOLD technique readily detects alterations in the concentration of deoxygenated blood and has demonstrated promise in quantifying the intrarenal oxygenation by the fact that a decreased R2* implies an increase in the pO_2 both within blood and renal tissue. This study demonstrated that the pO_2 level in the renal cortex and medulla correlated linearly with the pO_2 of arterial blood, although the slopes differed distinctly. Furthermore, the relationship between intrarenal $\Delta R2^*$ measured by BOLD and ΔpO_2 measured directly from oxygen-sensitive microelectrodes was defined for an inhaled fraction of oxygen in the range 5% to 70% (cortex $\Delta R2^*/\Delta pO_2 = -1.2\ msec^{-1}\cdot kPa^{-1}$ and medulla $\Delta R2^*/\Delta pO_2 = -1.7\ msec^{-1}\cdot kPa^{-1}$). This observation indicated that the measured R2* in the renal cortex did not change at the same rate as R2* in the renal medulla, presumably explained by the marked difference in blood supply to renal cortex and medulla. It is, however, surprising that the difference in $\Delta R2^*/\Delta pO_2$ ratio between the two regions are relative small taking into account that the saturation-pO_2 relationship of the medulla falls in the linear regime of the oxygen saturation curve while the cortex lies on the shoulder. This means that the sigmoid relationship of pO_2 to the oxygenation of hemoglobin (the oxygen-hemoglobin dissociation curve) makes the BOLD technique more sensitive to oxygen changes at low pO_2 levels. The lack of significant difference between the cortical and medullary $\Delta R2^*/\Delta pO_2$ ratio may be explained by the fact that the hematocrit influences the affinity of hemoglobin for oxygen and the difference in capillary and large vessels hematocrit will generate a shift in the oxygen-hemoglobin dissociation curve at these sites.

However, some important limitations associated with interpretation BOLD MRI data in terms of quantitative oxygen content must be addressed. Although this study demonstrated a linear relationship between R2* and pO_2 in the kidney based on BOLD MRI and microelectrode data from six presumably comparable normal pig kidneys, extrapolation to other subjects may likely suffer by different constraints associated with measurements of R2*. Although BOLD MRI is generally known to exploit the fact that R2* varies dependent on whether hemoglobin is in the oxygenated or in the deoxygenated form, the rate of spin dephasing is critically dependent on physical conditions such as magnet field inhomogeneity. In addition, Boxerman et al [20] demonstrated that R2* is influenced by the volume fraction of blood, vessel geometry, and applied pulse sequence parameters, supporting the view that the absolute magnitudes of R2* are less important than the relative changes. This goes along with

the findings revealed by Li et al [21], demonstrating a marked difference in R2* between individual subjects investigated at different times. Therefore, even though the linear relationship between R2* and pO_2 (as shown in this study) exists, R2* cannot be directly translated to pO_2. However, changes in R2* can be interpreted to be closely related to changes in pO_2 provided the measurements are made in the same regions before and after a physiologic/pharmacologic maneuver. Having said that R2* is in its nature related to the additional dephasing arising from an inhomogeneous static magnetic field, introduction of microelectrodes and electric cables into the magnet will cause local magnetic susceptibility variations. Hence, measurements of R2* was performed contralaterally to the kidney having inserted microelectrodes to avoid susceptibility artefacts induced by these devices.

R2* is lower in the renal medulla than in the renal cortex

In the present study we demonstrated that R2* is lower in the renal cortex as compared with R2* in the renal medulla. This confirms the findings of previous studies [7, 22], and the results indicated a lower oxygen supply or a high oxygen consumption in the renal medulla. Thus, the measured R2* values are comparable with those previously reported in humans [7, 21], BOLD MRI is critically dependent on the level of microscopic field inhomogeneity (i.e., reducing the voxel size will reduce the sensitivity because the level of inhomogeneity within a smaller volume element is obviously smaller). In other words, R2* values measured in the same region with significantly different voxel size will be different. As a consequence, R2* values cannot easily be compared in situations where different geometric parameters are being used.

The medulla includes different anatomical components all having limited oxygen availability [23], including the outer and the inner stripe of outer medulla as well as the inner medulla. The balance between oxygen demand and supply is most critical in the outer stripe where the tissue pO_2 is dependent on the active transport activity related to the Na^+, K^+-ATPase located in the thick ascending limb of the loops of Henle. Recently, these three intrarenal compartments have been separated in the rat kidney using BOLD MRI with distinct R2* values [24], demonstrating the highest R2* being located in the outer stripe of the outer medulla. This is consistent with the outer stripe being the site, which has the highest rate of oxygen consumption. In the present study, we did not distinguish between these renal medullary compartments because the multipapillary configuration of the pig kidney precluded the applied MRI protocol to provide a sufficient spatial resolution for this. Thus, differences between the different medullary zones could not be detected in the present study.

The MRI BOLD technique allows measurements of ΔR2*, but importantly is unable to distinguish differences in pO_2 levels produced by changes in the supply of oxygen (e.g., arterial blood supply) to the organ from those produced by changes in oxygen consumption by the tissue [7]. Obviously, supply of oxygen to the kidney is primarily dependent on the RBF and blood volume. The ability to sustain RBF under normoxic and hyperoxic conditions was recently demonstrated, under conditions with different concentrations of inhaled oxygen in conscious rats [25]. Conversely, it was found that hypoxia was associated with a generalized increase in RBF [25]. These findings indicate that even during conditions with hypoxia RBF may increase to a level where renal tissue pO_2 is maintained. In contrast, conditions with renal hypoperfusion may also be associated with an improved local oxygen supply to the outer medulla [25]. This takes place because of efferent arteriolar vasodilation, which causes an increase in RBF and a reduction in GFR [26]. On the other hand, it has been shown that an acute 60% reduction of RBF induced by renal arterial stenosis produced renal hypoxia predominantly in the cortex measured with R2* [27], supporting the ambivalent complexity of renal hypoxia.

Furosemide increases renal pO_2

The increase in medullary pO_2 (evidenced by an decreased R2*) in response to furosemide observed in healthy pigs in the present study is consistent with previous studies using BOLD MRI [28] and invasive oxygen-sensitive microelectrodes [26]. The decreased medullary R2* in response to furosemide is the result of an inhibition of the bumetanide sensitive cotransporter NKCC2 and thereby the reduction in active reabsorptive transport in the medullary thick ascending limb. Importantly, Dobrowolski, Badzynska, and Sadowski [29] demonstrated using laser Doppler technique a decrease in cortical and medullary blood flow in response to furosemide. Thus, the decreased R2* in the renal medulla as indicated by BOLD MRI in response to furosemide is consistent with a substantial decrease in oxygen consumption in the renal medulla.

The effect of UUO and release of UUO on intrarenal pO_2

The present study demonstrated that 24 hours of UUO slightly decreased R2* in the renal medulla, supporting the view that the metabolic work in the medulla is reduced, supposedly accompanied by the reduction in renal medullary blood flow, which has previously been demonstrated in response to acute UUO [30]. However, the pathophysiologic mechanisms involved in the renal hemodynamic and cellular changes in response to urinary tract obstruction are incompletely understood [13].

Regulation of oxygen levels in the renal medulla during UUO is complex and involves regulation of both oxygen supply and demands. Characteristically, the observed increase in $R2^*$ in the cortex after 24 hours of UUO supports the view that RBF and GFR are reduced in response to UUO, and secondary, there is a reduction of the filtered load to the tubules [31]. At the same time, renal pelvic and interstitial pressure is increased which may directly affect renal cellular mechanisms by stimulating apoptosis [32]. Previous studies have also demonstrated that obstruction is associated with transport defects in both the inner medulla and the collecting duct [33]. Studies in intact cells revealed marked reductions in the inhibitory effects of both furosemide and ouabain on oxygen consumption in cells from obstructed kidneys as compared with control animals, indicating reduced activities of both the Na, K, 2 Cl cotransporter and the Na, K, ATPase [34]. Consistent with this, in vivo studies demonstrated that UUO and release of UUO are associated with a marked down-regulation of key renal sodium transporters, especially the NKCC2 located to the thick ascending limb [35], suggesting that during UUO the demand for oxygen in the renal medulla is reduced. Furthermore, oxygen consumption studies revealed marked reductions in both the amiloride-sensitive and ouabain-sensitive QO2 in intact inner medulla collecting duct cells from obstructed kidneys, indicating a reduction in oxygen-dependent transport activities of both epithelial sodium channels and the Na, K, ATPase in the collecting duct [34]. These findings support the view that the renal medullary metabolism is highly susceptible to urinary tract obstruction. Consistent with this, the results of the present study indicated that the renal medullary oxygen content changes after UUO and importantly indicated an inappropriate relationship between reduction in demand and supply of oxygen. The reduction in RBF to the renal medulla and supply of oxygen cannot be compensated for by the reduction in metabolic oxygen demands.

The unchanged level of $R2^*$ at 24 hours after onset of UUO indicates a parallel reduction in the supply and demand for oxygen in the renal cortex during obstruction. Numerous energy-requiring processes take place in the renal cortex, in particular those related to epithelial cells of the tubules where reabsorption of solute and water takes place. During UUO most of the transport proteins involved in transepithelial transport of ions and water are down-regulated [36], supporting the view that a there is a general reduction in the demand for oxygen to drive the metabolic work.

CONCLUSION

Based on simultaneous measurements of $R2^*$ by BOLD MRI and pO_2 levels by oxygen sensing microelectrodes we were able to translate these parameters into unique conversion constants for the renal cortex and medulla, allowing us to relate $\Delta R2^*$ in terms of ΔpO_2. In addition, quantitative interpretation of $\Delta R2^*$ was conducted in two separate clinical relevant studies including measurements of intrarenal oxygenation after administration of furosemide and in response to 24 hours of UUO. The BOLD MRI technique offers a unique way to measure dynamic and regional changes in tissue oxygenation noninvasively. Future studies may successfully introduce BOLD MRI to evaluate oxygenation dependent mechanisms of nephrotoxicity and other conditions associated with chronic renal function impairment. The major limitation of this technique is that it cannot distinguish between alterations in renal oxygen supply and alterations in tissue oxygen consumption. It should also be emphasized that BOLD MRI is inherently complicated by different aspects including magnetic susceptibility effects, vessel geometry, and pulse sequence parameters, and measurements of $R2^*$ should therefore be interpreted only on a relative basis.

ACKNOWLEDGMENT

This study was performed by financial support from the Danish Medical Research Council.

Reprint requests to Jørgen Frøkiær, M.D., D.M.Sc., Professor of Clinical Physiology, The Water and Salt Research Centre/Clinical Institute, University of Aarhus, Aarhus University Hospital, Skejby, 8200 Aarhus N, Denmark.
E-mail: jf@ki.au.dk
Michael Pedersen, M.Sc., Ph.D., MR Research Centre Aarhus University Hospital, Skejby, Brendstrupgaardsvej 100, 8200 Aarhus N, Denmark.
E-mail: michael@mr.au.dk

REFERENCES

1. James PE, Bacic G, Grinberg OY, *et al*: Endotoxin-induced changes in intrarenal pO_2, measured by in vivo electron paramagnetic resonance oximetry and magnetic resonance imaging. *Free Radic Biol Med* 21:25–34, 1996
2. Silver IA: Problems in the investigation of tissue oxygen microenvironment. *Adv Chem Sev* 118:343–351, 1973
3. Liss P, Nygren A, Revsbech NP, Ulfendahl HR: Measurements of oxygen tension in the rat kidney after contrast media using an oxygen microelectrode with a guard cathode. *Adv Exp Med Biol* 411:569–576, 1997
4. Lui KJ, Gast P, Moussavi M, *et al*: Lithium phthalocyanine: A probe for electron paramagnetic resonance oximetry in viable biological systems. *Proc Natl Acad Sci USA* 90:5438–5442, 1993
5. Gullichsen E: Renal perfusion and metabolism in experimental endotoxin shock. *Acta Chir Scand* 560 (Suppl):7–31, 1991
6. Robertson PW, Hart BB: Assessment of tissue oxygenation. *Respir Care Clin N Am* 5:221–263, 1999
7. Prasad PV, Edelman RR, Epstein FH: Noninvasive evaluation of intrarenal oxygenation with BOLD MRI. *Circulation* 94:3271–3275, 1996
8. Gomori JM, Grossman RI, Yu-Ip C, Asakura T: NMR relaxation times of blood: Dependence on field strength, oxidation state, and cell integrity. *J Comput Assist Tomogr* 11:684–690, 1987
9. Lassen UV, Thaysen JH: Correlation between sodium transport and oxygen consumption. *Biochim Biophys Acta* 47:616–618, 1961
10. Koivisto A, Pittner J, Froelich M, *et al*: Oxygen-dependent inhibition of respiration in isolated renal tubules by nitric oxide. *Kidney Int* 55:2368–2375, 1999

11. Liss P, Nygren A, Revsbech NP: Intrarenal oxygen tension measured by a modified Clark electrode at normal and low blood pressure and after injection of x-ray contrast media. *Pflügers Arch* 434:705–711, 1997
12. Skolnok J, Takacs L, Szende E: In-vivo oxygen consumption of slices from kidney, brain, cortex and liver in hypoxia. *Nature* 209:305, 1966
13. Klahr S: Urinary tract obstruction, in *Diseases of the Kidney and Urinary Tract* (vol. 1), 7th ed., edited by Schrier RW, Philadelphia, Lippincott Williams & Wilkins, 2001, pp 757–787
14. Yanagisawa H, Jin Z, Kurihara N, *et al*: Increases in glomerular eicosanoid production in rats with bilateral ureteral obstruction are mediated by enhanced enzyme activities of both the cyclooxygenase and 5-lipoxygenase pathways. *Proc Soc Exp Biol Med* 203:291–296, 1993
15. Chou SY, Porush JG, Faubert PF: Renal medullary circulation: Hormonal control. *Kidney Int* 37:1–13, 1990
16. Frøkiær J, Nielsen AS, Knudsen L, *et al*: Enhanced intrarenal angiotensin II generation in the renal hemodynamic response to acute unilateral complete obstruction of the pig ureter. *Am J Physiol* 263:F527–F533, 1992
17. Frøkiær J, Nielsen AS, Knudsen L, *et al*: The effect of indomethacin infusion on renal hemodynamics and on the renin-angiotensin system during unilateral ureteric obstruction of the pig. *J Urol* 150:1557–1563, 1993
18. Reilly AM, Harris PJ, Williams DA: Biphasic effect of angiotensin II on intracellular sodium concentration in rat proximal tubules. *Am J Physiol* 269:F374–F380, 1995
19. Norman JT, Stidwill R, Singer M, Fine LG: Angiotensin II blockade augments renal cortical microvascular pO_2 indicating a novel, potentially renoprotective action. *Nephron Physiol* 94:39–46, 2003
20. Boxerman JL, Hamberg LM, Rosen BR, Weisskoff RM: MR contrast due to intravascular magnetic susceptibility perturbations. *Magn Reson Med* 34:555–566, 1995
21. Li LP, Storey P, Pierchala L, *et al*: Evaluation of the reproducibility of intrarenal $R2^*$ and delta $R2^*$ measurements following administration of furosemide and during waterload. *J Magn Reson Imaging* 19:610–616, 2004
22. Prasad PV, Priatna A: Functional imaging of the kidneys with fast MRI techniques. Review. *Eur J Radiol* 29:133–148, 1999
23. Rosen S, Epstein FH, Brezis M: Determinants of intrarenal oxygenation: Factors in acute renal failure. *Ren Fail* 14:321–325, 1992
24. Ries M, Basseau F, Tyndal B, *et al*: Renal diffusion and BOLD MRI in experimental diabetic nephropathy. *J Magn Reson Imaging* 17:104–113, 2003
25. Flemming B, Seeliger E, Wronski T, *et al*: Oxygen and renal hemodynamics in the conscious rat. *J Am Soc Nephrol* 11:18–24, 2000
26. Brezis M, Agmon Y, Epstein FH: Determinants of intrarenal oxygenation I. Effects of diuretics. *Am J Physiol Ren Physiol* 267:F1059–F1062, 1994
27. Juillard L, Lerman LO, Kruger DG, *et al*: Blood oxygen level–dependent measurement of acute intra-renal ischemia. *Kidney Int* 65:944–950, 2004
28. Epstein FH, Prasad P: Effects of furosemide on medullary oxygenation in younger and older subjects. *Kidney Int* 57:2080–2083, 2000
29. Dobrowolski L, Badzynska B, Sadowski J: Differential effect of frusemide on renal medullary and cortical blood flow in the anaesthetised rat. *Exp Physiol* 85:783–789, 2000
30. Sweeney P, Young LS, Fitzpatrick JM: An autoradiographic study of regional blood flow distribution in the rat kidney during ureteric obstruction—The role of vasoactive compounds. *BJU Int* 88:268–272, 2001
31. Hvistendahl JJ, Pedersen TS, Jørgensen HH, *et al*: Renal hemodynamic response to gradated ureter obstruction in the pig. *Nephron* 74:168–174, 1996
32. Miyajima A, Chen J, Poppas DP, *et al*: Role of nitric oxide in renal tubular apoptosis of unilateral ureteral obstruction. *Kidney Int* 59:1290–1303, 2001
33. Hwang SJ, Harris HW, Jr., Otuechere G, *et al*: Transport defects of rabbit inner medullary collecting duct cells in obstructive nephropathy. *Am J Physiol* 264:F808–F815, 1993
34. Hwang SJ, Haas M, Harris HW, Jr., *et al*: Transport defects of rabbit medullary thick ascending limb cells in obstructive nephropathy. *J Clin Invest* 91:21–28, 1993
35. Li C, Wang W, Kwon TH, *et al*: Altered expression of major renal Na transporters in rats with unilateral ureteral obstruction. *Am J Physiol Renal Physiol* 284:F155–F166, 2003
36. Li C, Wang W, Knepper MA, *et al*: Downregulation of renal aquaporins in response to unilateral ureteral obstruction. *Am J Physiol Renal Physiol* 284:F1066–F1079, 2003